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In-vitro study on the effect of pesticides on neuronal activity

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Abstract: Experiments have been conducted to examine the effect of chronic administration of bromoxynil, fluroxipir and bensultap on the in-vitro seizure susceptibility (induced by 4-aminopyridine) and excitability of neocortical slices of rat brain. The treatment regimes were (A) administration of spray solution in place of drinking water for seven days, and (B) feeding wheat which had been sprayed at growth stage Feekes 9–10 and consumed four to six weeks after spraying. The latency of appearance of the first seizure was significantly increased by fluroxipir (B) bensultap (B) and bromoxynil (A&B). Fluroxipir (A&B) decreased the frequency of seizure, and fluroxipir (A) and bensultap (B) doubled the duration of seizures. Excitability following electrical stimulation of the corpus callosum was not significantly changed by any treatments. The changes in brain activity were not related to the residue levels of the pesticides in the rat brains. Our results suggest that these chemicals may alter the functional properties of neuronal network activity and neurotransmission in rat neocortex after environmental exposure.

Keywords: bensultap; bromoxynil; fluroxipir; neuronal activity; brain slice; neurotoxicity

1 INTRODUCTION

The human population is exposed to toxic effects of various environmental chemicals including pesticides. Therefore it is important to learn more about the mechanisms of low-level or accidental exposure to, and potential biological effects of these compounds. To date cumulative, irreversible effects such as carcinogenesis and neurodegeneration have received the most attention. Histological and biochemical investigations have shown that different pesticides may cause several types of abnormality in the immune or the reproductive system.[1] However, there is relatively little information concerning pesticide-induced functional changes in the central nervous system.

There is a relatively large body of evidence concerning the neurophysiological effects of organophosphorus compounds,[2–4] chlorinated hydrocarbons[5–7] and certain pyridine compounds in mammals.[8,9] These types of pesticide may disturb normal neurotransmission, induce convulsions,[7,10] inhibit enzymes[11,12] or they can induce apoptotic cell death.[13] It is well known that organophosphorus pesticides (eg chlorpyrifos) exert their effect through inhibition of acetylcholinesterase.[2–4] Another group, chlorinated hydrocarbons (eg lindane), increase the excitability of the nervous system, causing severe epileptic discharges or neurodegeneration. These compounds act on the $GABA_A$ receptor.[5–7] However, only sporadic data are available about the neurotoxic effects of other types of agrochemical in wide use.

In our experiments we have studied three frequently used agrochemicals. Neurophysiological effects of the compounds have not been adequately examined. The chronic, low-dose exposure of bromoxynil caused severe behavioural alterations in dogs and humans,[14] but there was no such detectable effect in rats. The other two pesticides studied have not been examined for neurophysiological and behavioural parameters. In our present neocortical slice experiments, the effects of chronic pesticide application on neuronal excitability as well as on seizure susceptibility were analysed to estimate many neurotoxic potential. The field responses evoked by electrical stimulation and spontaneous seizure activity developed in 4-aminopyridine (4-AP)-containing solution were analysed in detail in neocortical slices of control and pesticide-treated rats. We have chosen subtoxic exposure regimes that caused no mortality or illness.

2 EXPERIMENTAL METHODS AND MATERIALS

2.1 Pesticide exposure

The experiments were performed on adult Wistar rats of both sexes (120–160 g, LATI, Gödöllô). The pesticide-treated and control groups consisted of three and 12 animals respectively. The compounds were administered for seven days. The animals received the pesticides either dissolved in the drinking water at a defined dose (see below), or they were fed with wheat pre-treated with the pesticides. The concentrations in the drinking water corresponded to the standard crop-spraying concentration of each pesticide. Total volume

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of consumption was measured. For feeding we used a wheat variety widely grown in Hungary (cv 'Martonvásári 21'). Wheat was sprayed with each pesticide early in summer during flowering (stadium: Feekes 9–10), and consumed four to six weeks after spraying.[15] The drinking experiment served as a model of accidental spraying exposure, and the feeding of treated wheat as a model of low-level chronic exposure during the milling process.

The following pesticides were applied:

Bromoxynil ('Pardner'; 3,5-dibromo-4-hydroxybenzonitrile), herbicide: 130 mg litre^{-1} solution, total consumption: ~200 ml per animal;
Fluroxipir ('Starane'; 4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid), herbicide: 72 mg litre^{-1} solution, total consumption: ~200 ml per animal;
Bensultap ('Bancol'; *S,S'*-[2-dimethylamino-trimethylene]bis-benzene-thiosulfonate), insecticide: 250 mg litre^{-1} solution, total consumption: ~200 ml per animal.

2.2 Slice method and electrophysiology

The brain was removed from rats under deep ether anaesthesia. A tissue block containing the somatosensory cortex was cut out and coronal slices (400 μm) were prepared with a vibrotome. Slices were incubated in HEPES-containing solution (pH = 7.0) before putting them into the interface type recording chamber, which was continuously perfused (3 ml min)$^{-1}$ with the standard perfusion solution containing (in mM): 126 NaCl; 1.8 KCl; 1.25 KH_2PO_4; 1.3 $MgSO_4$; 26 $NaHCO_3$; 2.4 $CaCl_2$; 10 glucose, saturated with carbogene (5% CO_2-95% O_2). Recordings were performed at 33 °C. Extracellular glass recording electrodes (8–10 MΩ) were filled with 1 M NaCl and positioned in the middle layer of the cortex. The bipolar platinum electrodes used for stimulation were positioned above the recording electrodes at the border of the white and grey matter.

Stimulus intensity dependence of evoked responses was first measured in standard perfusion solution. By stimulating the corpus callosum, the threshold of field potentials and the stimulus intensity giving maximal response were determined. The amplitude and the duration of evoked responses were also measured. Subsequently, the spontaneous seizures developed as the consequence of 60 min 60 μM 4-AP application were characterised. The latency of the appearance of first seizure as well as the frequency and duration of fully developed spontaneous discharges were analysed.

Statistical analysis was performed on the data by Student's unpaired *t*-test (*$P < 0.05$) between control and each treated groups.

3 RESULTS

No spontaneous activity was detectable in any of the slices investigated in standard perfusion solution. In the solution containing 4-aminopyridine, epileptic activity developed and stabilised within 60 min (Fig 1). The latency of appearance of the first spontaneous event was longer in each pesticide-treated group than in the control, with significant differences in four cases (Table 1). The frequency of epileptic discharges was reduced in all cases, with significant changes following fluroxipir exposure, where the average decrease of frequency was about 68%. Generally, a considerable increase of duration of each discharge was observed in parallel to the decrease of frequency. The changes were the most significant in the case of fluroxipir exposure in drinking water (86%) and bensultap exposure with pre-treated wheat (100%).

Figure 1. Original record of spontaneous activity developed in 4-aminopyridine solution (60 μM). Calibration: 0.5 mV and 10 s.

Table 1. Parameters of spontaneous activity developed in rat neocortical slices in response to 4-aminopyridine (60 μM)

Treatment	*Latency of first seizure (min)*	*Frequency of seizures (min^{-1})*	*Duration of seizures (ms)*
Control	6.5 (±0.8)	4.6 (±0.9)	552 (±63)
Fluroxipir (A)[a]	8.33 (±3.33)	1.6 (±0.41)*	1025 (±54)*
Fluroxipir (B)[b]	24.0 (±4.64)*	1.4 (±0.41)*	647 (±162)
Bensultap (A)	15.66 (±9.68)	3.0 (±0.4)	659 (±54)
Bensultap (B)	30.0 (±12.58)*	2.73 (±0.17)	1107 (±183)*
Bromoxynil (A)	14.0 (±5.34)*	3.33 (±0.33)	754 (±169)
Bromoxynil (B)	23.0 (±2.0)*	2.2 (±0.6)	735 (±204)

[a] (A) pesticide application in drinking water.
[b] (B) feeding with pre-treated wheat.
* Significant differences ($P < 0.05$, Student's *t*-test) between control and pesticide-exposed slices.

The parameters of evoked field responses were characterised in standard perfusion solution (Fig 2). Threshold values of stimulation intensity necessary to evoke responses in the treated groups did not significantly differ from control data. The maximal amplitude and the duration of evoked responses were variable, depending on the pesticide and the type of exposure. No significant differences were detectable among the control and treated groups in any parameter (Table 2).

The total quantity of pesticide in treated and control rat brains was analysed by GC. Considerable brain accumulation of two pesticides (bensultap and bromoxynil) was detected parallel to our neurophysio-

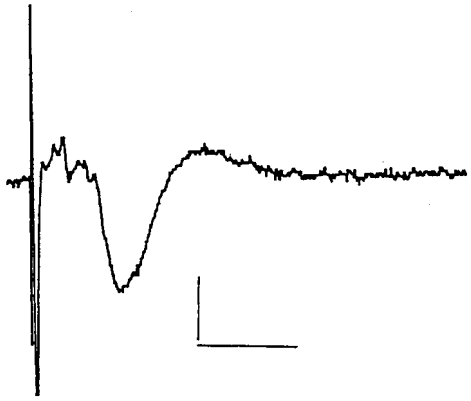

Figure 2. Original record of an evoked field response in standard perfusion solution. Calibration: 0.25 mV and 10 ms.

Table 2. Characteristics of evoked field potentials in neocortical slices from control and pesticide-exposed rats

Treatment	*Threshold of field response (V)*	*Duration of field response (ms)*	*Amplitude of field response (mV)*
Control	3.7 (±0.6)	25.0 (±2.5)	0.86 (±0.04)
Fluroxipir (A)[a]	3.16 (±0.17)	23.33 (±3.33)	0.85 (±0.12)
Fluroxipir (B)[b]	3.125 (±0.37)	25.0 (±2.04)	0.92 (±0.23)
Bensultap (A)	3.66 (±0.44)	28.33 (±6.41)	0.63 (±0.19)
Bensultap (B)	3.66 (±0.16)	23.33 (±3.33)	0.75 (±0.09)
Bromoxynil (A)	4.25 (±0.43)	17.5 (±3.23)	0.62 (±0.16)
Bromoxynil (B)	2.75 (±0.25)	25.0 (±5.0)	0.76 (±0.22)

[a] (A) application in drinking water.
[b] (B) feeding with pre-treated wheat.

Table 3. Residues of pesticides in rat brain following treatment

Compound	*Treatment*	*Residue ($\mu g\,g^{-1}$)*[a]
Fluroxipir	A[b]	nd
Fluroxipir	B[c]	nd
Bensultap	A	6.28
Bensultap	B	1.84
Bromoxynil	A	0.27
Bromoxynil	B	0.11

[a] nd = non-detected. Limit of detection was: 0.06 $\mu g\,g^{-1}$.
[b] (A) application in drinking water.
[c] (B) feeding with pre-treated wheat.

logical examinations. The accumulation was greater when the pesticide was applied in the drinking water than from the consumption of pre-treated wheat. The accumulation of fluroxipir in the brains of treated animals was under the limit of detection (Table 3).

4 DISCUSSION

Our data provide the first evidence concerning the neuronal effect of fluroxipir and bensultap. Based on brain residue levels we suggest that bromoxynil and bensultap may cross the blood–brain barrier and are able to exert an effect directly on the nervous tissue. However, fluroxipir content of the brain was under the limit of detection, and we have not examined the metabolism and the accumulation of its derivatives in the brain. In spite of this fact, we were able to detect the effect of fluroxipir in the neurophysiological measurements. This means that this type of physiological investigation may be useful to indicate the effects of chemicals. The electrophysiological results indicate that chronic exposure of the three pesticides examined caused changes both in the excitability and in the spontaneous seizure activity in the neocortex. We observed a mild inhibitory effect both on spontaneous firing and on evoked responses. Further investigations are require to determine the exact mechanism of these effects.

We think that the examination of the possible effects of sub-toxic doses of agrochemicals in widespread use on brain tissue from mammals is very important in relation to risk assessment and the protection of the public. We hope that these types of investigations may be useful for the selection of the less toxic pesticides from amongst those which have similar effects from an agricultural point of view.

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